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Planting dates and plant densities influence on morpho-physiological responses, forage productivity and nutritive value of clitoria in an arid region

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ABSTRACT

Clitoria (*Clitoria ternatea* L.) is a perennial multi-purpose legume plant could provide high-value forage for livestock in the arid regions such as Egypt. There is little knowledge concerning the potential of forage clitoria as an arable crop in such climates. A 2-year field experiment (2015/2016) was carried out to investigate the influence of two planting dates on May 20 as early (P_E) and June 20 as late (P_L) combined with three plant densities (33.33=D_{10}, 22.22=D_{15}, and 16.66=D_{20} plants m^{-2})on clitoria's morpho-

physiological responses, forage productivity, and nutritive value. Delay planting decreased cumulative fresh forage yield CFFY by 14.4% and 19.5% and cumulative dry forage yield CDFY by 9.4% and 18.2% in both seasons, respectively, whereas cumulative protein yield CPY decreased by 20.5% in the 2nd season compared with early planting. Clitoria responded significantly to change plant density, lower density (D_{20}) had the highest morpho-physiological attributes except for plant height (PH), leaves area index (LAI), leaf/stem ratio (LSR), and membrane stability index (MSI).While, higher density (D_{10}) yielded the greatest CFFY (23.3 and 23.1 t ha^{-1}),CDFY (7.5 and 7.4 t ha^{-1}) and CPY (1.55 and 1.39t ha^{-1}) compared with those others in both seasons, respectively. Clitoria planted early (P_E) under high density of D_{10}had higher almost all morpho-physiological responses and productivity besides protein content in both seasons. It could deduce that the application of early planting date (P_E) combined with high density of D_{10} is the most adequate combination for producing the highest forage productivity with acceptable nutritive value of clitoria in an arid environment.

Keywords: Forage clitoria; morpho-physiological responses; nutritive value; plant densities; planting dates; arid regions.

1. INTRODUCTION

Clitoria (*Clitoria ternatea* L.) is a warm-season plant, belongs to the family *Fabaceae*, perennial multipurpose legume forage native to tropical America (Ramakrishnan et al. 2018), provides bioactive compounds for curative use (Mukherjee et al. 2008). The green color of leaves is added to food and the bright blue color of flowers to rice cakes in Malaysia. Thanks to its attractive flower colors, it is grown as an ornamental plant. It exhibits excellent regrowth after cutting or grazing within short period, high nutritive value, thin stem, large leaves, higher leaf protein content, lower acid digestible fiber, non-toxic and nil bloat which make it optimal plant for fresh forage and hay or silage making (Barros et al. 2004). As well, it is grown as a cover crop, green manure crop (Gomez and Kalamani 2003) and improving soil fertility by atmospheric N_2 fixation capability to enhance yields of subsequent crops (Sánchez et al. 2011). Moreover, clitoria is a highly palatable and preferred by livestock such as sheep, goat and cattle over other most tropical forage legumes. Moreover, it is considered low to moderate tolerance to soil salinity and can grow to 50% of maximum growth at EC_e 6.4 dS m^{-1} (Keating et al. 1986). Despite of the clitoria is a short-day C_3 plant, it is grows vigorously in full sunlight but moderately shade tolerant (Sanchez and Ibrahim 1991). In view of these features, there are great efforts to introduce and boost the legume forage plants such as clitoria in the Egyptian agricultural system (Abdelhamid and Gabr 1993).

Egypt, as a developing country located in the arid and semi-arid regions, faces four major challenges for sustainable agricultural development, namely high rate of population growth, limited natural resources of fresh water, existence of salt affected soils and shortage of food and feed (Mohamed et al. 2018). Thus, studies should be oriented towards solving these problems giving particular attention to expansion in cultivation of newly reclaimed soils in an attempt to achieve self-sufficiency in various agricultural products, whereas agriculture contributes about one-seventh of the gross domestic product and employs nearly one-fourth of the Egyptian labor force (Egypt in Figures 2015). From the other side, livestock offer various products and services particular for rural population, low quantity and quality of feed besides low genetic potential have been reported as major factors associated with livestock performance (Descheemaeker et al. 2010). Apparently, Egypt also suffers from an acute shortage of forage crops, particularly during the summer season. The reasons for this problem were due to that Egypt does not have natural pastures, relative stability of the area that devoted to production of forage crops mainly owing to the strong competition between human and ruminant animals on the limited arable land in addition to the concentrated feedstuffs are very expensive (Mahfouz et al. 2015). Annually, Egypt cultivates about 296.8 thousand ha of summer and autumn forage crops besides alfalfa and silage crops represents 10.3% of the total area of summer and autumn crops in 2015 season (Egypt in Figures 2015).

In this respect, clitoria might be very promising legume forage plant in the future especially in the newly reclaimed soil as an arid environment. Improving forage productivity and nutritive value are considered to be principal goals for ruminant animal breeders (Carmi et al. 2006). Forage quantity and nutritive value of clitoria as other forages vigorously depends on genotype and several environmental factors such as climatic conditions, plant density, timing of beginning cutting, cutting intervals, availability of water and mineral nutrition in soil, etc. Planting time is a very important non-monetary factor that determines the yield of clitoria (Ramanjaneyulu et al. 2018). Optimal planting date of any crop may differ according to cultivar, region and sometimes season because of the great variation of agro-ecological conditions (Sarkar et al. 2004). Selection of the appropriate planting date guarantees the perfect synchronization of each stage of plant growth and its suitable climatic conditions during developmental stages to achieve a potential forage yield (Özel et al. 2004;Sim et al. 2015). As well as, plant density is an important factor related to quantitative and qualitative parameters in forage plant (Mattera et al. 2013; Ramanjaneyulu et al. 2018).Also, plant density has been shown to be effective in intercepting the solar radiation and therefore degree of dry matter accumulating in forage legumes (Purcell

et al. 2002).So, choosing the plant density is based on the hypothesis that the optimal density should enable the plant canopy to intercept the fully photosynthetically active radiation, resulting in higher yield. This response was noted in clitoria (Nawal 2001) and various crops such as soybean (Andrade et al. 2002), Lucerne (Mattera et al. 2009, 2013), corn (Ferreira et al.2014), and cowpea (Kamara et al. 2018).

In our best knowledge, very little scientific works have been reported on agronomic practices techniques and their effects on performance quality of clitoria till now. Therefore, the present investigation was designed to assess the integrative effect of planting dates and plant densities on morpho-physiological attributes, forage productivity and nutritive value indices for clitoria grown in dry environment such as newly reclaimed soil under El-Fayoum province conditions in Egypt.

2. MATERIALS AND METHODS

Experimental site, climatic data and soil properties

A 2-year field experiment (2015-2016) was conducted at Demo experimental farm of the Faculty of Agriculture which located in southeast El-Fayoum province (29.17° N; 30.53° E), Egypt. According to aridity index (Ponce et al. 2000), the region is located under dry climatic conditions. Also, the period from May to Sept. is generally hot and dry and there was no rainfall with average maximum daily temperature of 37.54 and 38.56°C, average minimum daily temperature of 22.54 and 23.10°C, average air relative humidity of 38.34 and 34.92% and average seasonal class "A" pan evaporation of 6.84 and 6.48 mm for the experimental area climate during the two growing seasons, respectively, as presented in Table 1. Before planting, soil samples were taken from 0 to 60 cm depth represents three layers and analyzed for selected physico-chemical features as shown in Table 2.

Table 1 Monthly averages of some climatic data at Fayoum region during experiment period in 2015 and 2016 growing seasons

Month	T_{max}(°C)*	T_{min} (°C)	T_{max} (°C)	T_{min} (°C)	RH_{avg} (%)		E_p (mm d^{-1})		SR_{avg} (MJ m^{-2} d^{-1})
	2015		2016		2015	2016	2015	2016	
May	36.9	21.6	36.1	19.9	39.7	31.4	6.9	7.1	14.4
June	36.0	21.7	40.3	24.4	42.0	34.5	7.3	7.5	15.4
July	37.0	21.8	39.3	23.9	37.6	36.6	6.9	6.9	14.3
Aug.	39.0	24.1	38.9	23.7	36.5	34.9	7.2	5.1	12.7
Sept.	38.8	23.5	38.2	23.6	35.9	37.2	5.9	5.8	11.4

*T_{max} and T_{min} are maximum and minimum air temperatures, respectively, RH_{avg} is average relative humidity, E_P is average of measured pan evaporation class A, and SR_{avg} is average of solar radiation.

Table 2 Some initial physical and chemical properties of soil at the experimental site as an average of two growing seasons.

Characteristic	Soil layer (cm) 0 – 20	20 – 40	40 - 60
	Physical properties		
Sand (%)	72.50	74.60	74.20
Clay (%)	14.60	13.40	13.70
Silt (%)	12.90	12.00	12.10
Soil texture	Sandy Loam	Sandy Loam	Sandy Loam
Bulk density (g cm^{-3})	1.46	1.57	1.58
Field capacity (%)	19.79	19.42	18.62
Wilting point (%)	6.69	3.64	4.37
Available water (%)	13.10	15.78	14.25
	Chemical properties		
Organic content (%)	0.90	0.82	0.65
$CaCO_3$ (%)	17.5	18.6	21.4

pH in 1:2.5 soil : water extract	7.86	7.78	7.92
EC in extracted soil paste (dS m^{-1})	3.70	3.40	3.10
Total N (mg kg^{-1})	14.81	13.24	13.21
Available P (mg kg^{-1})	3.25	3.28	3.15
Available K (mg kg^{-1})	42.57	39.87	39.24

Experimental design, treatments and crop husbandry practices

The experimental design was split plot in randomized complete blocks with three replicates. Two investigated factors including two planting dates *viz*., May 20 as early (P_E) and June 20 as late (P_L) were placed in main plots and three intra-ridge spacings, *viz*.,10, 15 and 20 cm among hills on one side of ridge, this achieve plant densities of 33.33 (D_{10}), 22.22 (D_{15}) and 16.66 (D_{20}) plants m^{-2}, respectively, were randomly allotted to subplots. Each subplot size was 3 by 3.5 m equal10.5 $m^2$and consisted of five rows 60 cm apart and 3.5 m long. One external ridge of each side was used as a guard and the three inner ridges were used for sampling and forage yield estimation.

Selected seeds of clitoria (*Clitoria ternatea* L. cv. Baladi) were obtained from the Sudanese Ministry of Agriculture. Immediately prior to planting clitoria seeds were inoculated with *Rhizobium* sp. of the cowpea group according to (Abreu et al. 2014) to fix atmospheric nitrogen. Uniform 3 to 5 seeds of clitoria were hands sown in hills at a depth of 4 cm. Seedlings were thinned to two fairly uniform per hill before the first irrigation.

Prior to sowing, 475 kg ha^{-1} of calcium superphosphate (15.5% P_2O_5) and 120 kg ha^{-1} potassium sulfate (48% K_2O) were incorporated into the soil during seedbed preparation. Clitoria plants failed to build root nodulations when their roots were checked after 30 days after planting (DAP). Therefore, clitoria plants received nitrogen fertilizer in the form of ammonium nitrate (33.5% N) at rate 71 kg ha^{-1} to each cut divided into two equal doses top dressed before the 2nd and 3rd irrigations in the 1st cut and added directly after cutting before 1st and 2nd irrigations in the 2nd cut in both growing seasons. Weeds control was done one to two times manually as per the requirement in each growth cycle.

Sampling, measurements and data collection

Clitoria plants were clipped two consecutive harvests during each growing season. The 1st one at the age of 60 DAP while the 2nd cutting was performed after 45 days from the 1st harvest. Also, all harvests were clipped manually by mower at a uniform stubble height of approximately 10 cm above ground (De Araujo Filho et al. 1994). Before fifteen days of cutting in each growth period, first sample of six individual plants were randomly chosen from one of the central three ridges of each sub plot. These plants were instantly carried to laboratory and were dried in an electric oven at 70±2^{0}C till constant weight to record dry weight (DW) of plants.

At harvest time in each cut, second sample composed of six individual plants were randomly chosen from the same ridge that was designated to select the first sample plants of each sub plot. These plants were instantly carried to laboratory to record the following attributes before dried it in an electric oven at 70±2^{0}C till constant weight to record DW of plants. Measured morpho-physiological attributes included plant height (PH cm), number of leaves/plant (NL/P), number of branches/plant (NB/P), plant dry weight (PDW g), leaf/stem ratio (LSR) was calculated based on fresh weight and leaves area index (LAI) was estimated as present in the following equation:

$$\mathrm{LAI} = [\text{Leaf area per plant}/\text{Ground area per plant}]$$

after using the punch method proposed by Semida et al. (2017) by taking forty uniform leaf discs by punching avoiding major leaf veins by a cork borer from fully expanded leaf tissue and calculated leaves area per plant in cm^2 using the following equation:

$$\text{Leaf area per plant (cm}^2) = (\text{Leaf area of discs} \times \text{Dry weight of leaves/plant})/(\text{Dry weight of leaf discs}$$

Also, total chlorophyll (Total chl. mg g^{-1}) was extracted and determined according to procedures presented by Arnon (1949). Relative water content (RWC%) was estimated adopting to the method of Hayat et al. (2007) using the equation:

$$\mathrm{RWC}\ (\%) = [(\mathrm{FW} - \mathrm{DW})/(\mathrm{TW} - \mathrm{DW})] \times 100$$

Membrane stability index (MSI%) was determined according to the method of Premachandra et al. (1990) using the equation:

$$\mathrm{MSI}\ (\%) = [1 - \mathrm{EC}_1/\mathrm{EC}_2] \times 100$$

Electrolyte leakage (EL%) was determined according to the method described by Lutts et al. (1996)defining as follows:

$$\mathrm{EL}\ (\%) = [\mathrm{EC}_1/\mathrm{EC}_2] \times 100.$$

Free proline (mg g^{-1} DW) was determined in dry powder samples using the rapid colourimetric method represented by Bates et al. (1973). Absolute growth rate (AGR g d^{-1}) between two different times in each growth cycle as mentioned above was calculated based on the dry matter accumulation by using the formula given by Hunt (1990):

$$\mathrm{AGR}\ (\mathrm{g\ d^{-1}}) = [(\mathrm{W}_2 - \mathrm{W}_1)/(\mathrm{T}_2 - \mathrm{T}_1)]$$

For nutritive value analysis, representative dried samples of clitoria leaves and stems were taken from each sub plot at harvest in each cut for forage analysis. These samples were grinded by a stein sample mill (Model M-2, The Steinlite Corporation, Kansas, USA) to fine powder and were finely mixed and passed through a 0.5 mm sieve. Near infrared reflectance spectroscopy (NIRs) apparatus (model DA1650, manufactured by FOSS Corporation at wavelength region from ~750 nm to 2500 nm of the electromagnetic spectrum) was used to assess the principal nutritive value indices in the powdered clitoria samples included protein, fiber and non-structural carbohydrates (NSC) percentages for each cut during the both seasons.

Fresh forage yield (t ha^{-1}) was appraised by cutting clitoria plants from the central two ridges of each sub plot (4.20 m^2) for each cut and weighed in kg then converted to t ha^{-1}, where dry forage yield (t ha^{-1}) was estimated by drying sub samples of fresh forage in an electric oven at 70±2°C till a constant weight and dry matter content (DM%) was computed and then dry forage yield (t ha^{-1}) was calculated by multiplying fresh forage yield (t ha^{-1}) by DM% for each cut, then it can be determine the cumulative fresh forage yield (CFFY t ha^{-1}) and cumulative dry forage yield (CDFY t ha^{-1}) by aggregating of fresh and dry forage yields for the two cuts during the full season. Also, cumulative protein yield (CPY t ha^{-1}) was computed by multiplying protein% by DM% for each cut, then aggregating of protein yield for the two cuts in full season.

Statistical analysis

All collected data for various parameters were statistically analyzed according to technique of analysis of variance for split plot arranged in randomized complete block design using the InfoStat computer software package version 2012 (Casanoves et al. 2012). Prior to analysis of variance, each attribute was explored for normal distribution agreeing to test of Shapiro-Wilk's (with confidence level $P \leq 0.05$ for significant difference from normal distribution).Most of the attributes were in normal or close by the normal distribution (i.e., the difference from a normal distribution was not significant; NS). The differences among treatment means were compared by Duncan's multiple range as a post hoc test (Gomez and Gomez 1984).

3. RESULTS

Morpho-physiological attributes

Planting dates had significant effects on all morpho-physiological attributes of clitoria plant in the two cuts in both seasons except for PH, PDW, MSI and AGR in the 2nd cut of the 1st season and total chl. in the 2nd cut of both seasons (Tables 3-6). Early planting date (P_E) produced the highest NL/P (42.1 and 171.3 & 37.8 and 148.6), NB/P (4.2 and 18.8 & 4.5 and 16.0), LAI (1.9 and 9.0&1.4 and 8.0), LSR (1.93 and 1.31& 1.76 and 0.82), and RWC (81.0 and 68.4 & 73.4 and 91.5%) in the two cuts of both seasons, respectively, whereas produced the highest PH (45.8 and 63.9 cm), PDW (4.4 and 24.1 g), MSI (64.9 and 85.6%) and AGR (0.19 and 0.67 g d^{-1}) in the two cuts, respectively, of the 2nd season. Also, it produced the highest PH (51.2 g), PDW (5.5 g), MSI (84.8%) and AGR (0.25 g d^{-1}) in the 1st cut of the 1st season besides total chl. in the 1st cut (16.0 and 20.0 mg g^{-1}) of both seasons, respectively. However, the greatest EL (82.3 and 60.6 & 91.8 and 89.1%) in the two cuts of both seasons, respectively, and free proline (2.7 and 1.1 mg g^{-1} DW) in the two cuts, respectively, of the 2nd season were obtained from clitoria that planted later.

For plant densities, changing of hill spacing within ridge had significant influence on all morpho-physiological attributes of clitoria in both seasons except for LSR and free proline in the 2nd cut during the 1st season also RWC and EL in the 2nd cut during the 2nd season. Clitoria plants grown under high density D_{10} had higher PH (51.3 and 58.1 & 45.3 and 61.8 cm) and LAI (2.1 and 9.0 & 1.5 and 8.8) for both cuts in the 1st and 2nd seasons, respectively, in addition LSR (1.69, 1.70 and 0.81) and MSI (83.2, 57.7 and 77.2%) for the 1st cut in the 1st season and both cuts in the 2nd season, respectively. Moreover, plants grown under low density D_{20} recorded the

highest NL/P (39.7 and 175.0 & 37.1 and 145.0), NB/P (3.9 and 18.8 & 4.6 and 16.6), PDW (5.6 and 24.8 & 4.6 and 24.4 g) and AGR (0.26 and 0.88 & 0.22 and 0.63 g d^{-1}) for both cuts in the 1st and 2nd seasons, respectively, else EL (68.6%) for the 1st cut and total chl. (12.8 mg g^{-1}) for the 2ndcut during the 1st season also free proline (2.3 and 1.1 mg g^{-1}) for both cuts, total chl. (16.9 mg g^{-1}) and EL (81.2%) for the 1st cut during the 2nd season. Whereas, the highest RWC (75.8 and 73.6%) for both cuts, total chl. (14.6 mg g^{-1}) and free proline (4.4 mg g^{-1}) for the 1st cut and EL (58.4%) for the 2nd cut during the 1st season, likewise RWC (70.6%) for the 1st cut and total chl. (18.5 mg g^{-1}) for the 2nd cut during the 2nd season were achieved at medium density D_{15}.

Table 3 Effects of planting dates, plant densities and their interaction on plant height (PH), number of leaves/plant (NL/P) and number of branches/plant (NB/P) for the two cuts of clitoria plant during 2015 and 2016 growing seasons.

Treatment	PH (cm)		NL/P		NB/P	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
	2015 season					
Planting date (P)	**	NS	**	**	**	**
Early (P_E)	51.2±2.8a	56.5±0.8a	42.1±2a	171.3±12a	4.2±0.14a	18.8±1.3a
Late (P_L)	39.0±1.2b	55.6±0.9a	25.7±2b	117.7±5b	2.6±0.18b	12.5±0.5b
Density (D)	**	**	**	**	**	**
D_{10}	51.3±3.6a	58.1±0.6a	27.0±3c	116.8±6c	2.9±0.35c	12.8±0.9c
D_{15}	45.9±3.5b	56.8±0.6a	35.1±5b	141.6±12b	3.4±0.40b	15.4±1.2b
D_{20}	38.2±1.2c	53.2±0.6b	39.7±3a	175.0±18a	3.9±0.29a	18.8±2.1a
P x D	**	NS	**	**	NS	**
	2016 season					
Planting date (P)	**	**	**	**	**	**
Early (P_E)	45.8±1.8a	63.9±1.1a	37.8±1.8a	148.6±5a	4.5±0.27a	16.0±0.7a
Late (P_L)	38.6±0.4b	53.4±1.0b	27.6±1.3b	116.2±4b	3.0±0.18b	13.6±0.5b
Density (D)	**	**	**	**	**	**
D_{10}	45.3±3.1a	61.8±2.7a	26.9±1.8c	117.3±5.6c	3.2±0.32b	12.9±0.5c
D_{15}	41.3±1.6b	58.1±2.2b	34.1±2.6b	134.9±10b	3.4±0.30b	14.8±0.4b
D_{20}	40.1±0.4c	56.0±2.4b	37.1±2.5a	145.0±6.2a	4.6±0.40a	16.6±0.8a
P x D	**	NS	*	**	NS	**

P_E and P_L indicate to planting on May 20 and June 20, respectively. *, ** indicate to the significant difference at $P \leq 0.05$ and $P \leq 0.01$, respectively; and "ns" indicates to non-significant difference. Means ± SE in each column followed by the same letter are not significantly different according to Duncan's multiple range test.

Table 4 Effects of planting dates, plant densities and their interaction on plant dry weight (PDW), leaves area index (LAI) and leaf/stem ratio (LSR) for the two cuts of clitoria plant during 2015 and 2016 growing seasons.

Treatment	PDW (g)		LAI		LSR	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
	2015 season					
Planting date (P)	**	NS	*	**	**	**
Early (P_E)	5.5±0.5a	22.4±1.0a	1.9±0.10a	9.0±0.54a	1.93±0.03a	1.31±0.01a
Late (P_L)	3.2±0.2b	21.6±0.7a	1.7±0.12b	5.3±0.53b	1.22±0.04b	0.92±0.02b
Density (D)	**	**	**	**	**	NS
D_{10}	3.2±0.2c	19.3±0.1c	2.1±0.06a	9.0±0.77a	1.69±0.15a	1.14±0.07a

D_{15}	4.3±0.6b	21.8±0.3b	1.9±0.16b	6.9±1.06b	1.48±0.17c	1.11±0.09a
D_{20}	5.6±0.8a	24.8±0.5a	1.4±0.06c	5.5±0.71c	1.56±0.16b	1.11±0.11a
P x D	**	*	**	**	NS	**
			2016 season			
Planting date (P)	**	**	**	**	**	**
Early (P_E)	4.4±0.3a	24.1±0.8a	1.4±0.11a	8.0±0.88a	1.76±0.08a	0.82±0.03a
Late (P_L)	3.2±0.2b	19.8±0.6b	1.1±0.05b	5.4±0.36b	1.28±0.01b	0.71±0.02b
Density (D)	**	**	**	**	**	**
D_{10}	2.8±0.3c	19.8±0.7c	1.5±0.13a	8.8±0.90a	1.70±0.16a	0.81±0.05a
D_{15}	3.9±0.3b	21.6±1.1b	1.2±0.09b	6.5±0.79b	1.40±0.07c	0.78±0.01b
D_{20}	4.6±0.3a	24.4±1.1a	1.0±0.02c	4.7±0.08c	1.47±0.09b	0.70±0.03c
P x D	NS	NS	**	**	**	**

P_E and P_L indicate to planting on May 20 and June 20, respectively. *, ** indicate to the significant difference at $P \leq 0.05$ and $P \leq 0.01$, respectively; and "ns" indicates to non-significant difference. Means ± SE in each column followed by the same letter are not significantly different according to Duncan's multiple range test.

Table 5 Effects of planting dates, plant densities and their interaction on total chlorophyll (total chl.), relative water content (RWC) and membrane stability index (MSI) for the two cuts of clitoria plant during 2015 and 2016 growing seasons.

Treatment	Total chl. (mg g^{-1})		RWC (%)		MSI (%)	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
			2015 season			
Planting date (P)	**	NS	**	*	*	NS
Early (P_E)	16.0±0.3a	12.3±0.4a	81.0±1.0a	68.4±4.7a	84.8±1.8a	46.7±2.1a
Late (P_L)	11.9±0.1b	11.8±0.2a	65.0±0.9b	63.6±0.7b	74.2±1.6b	41.0±0.8a
Density (D)	**	*	**	**	**	**
D_{10}	13.8±0.7b	11.7±0.3b	72.4±3.3b	57.3±2.7c	83.2±2.3a	43.0±2.7b
D_{15}	14.6±1.1a	11.8±0.4b	75.8±3.9a	73.6±4.3a	81.5±3.0a	42.8±1.7b
D_{20}	13.6±0.9b	12.8±0.4a	70.8±3.6b	67.0±2.0b	73.9±2.5b	45.8±2.4a
P x D	**	**	NS	**	NS	**
			2016 season			
Planting date (P)	**	NS	*	**	**	**
Early (P_E)	20.0±0.6a	17.4±0.4a	73.4±0.4a	91.5±0.9a	64.9±3.1a	85.6±1.2a
Late (P_L)	12.5±0.1b	16.3±0.5a	64.7±0.8b	72.7±0.9b	39.1±3.1b	61.9±1.3b
Density (D)	**	**	*	NS	**	*
D_{10}	15.0±1.3b	16.0±0.4b	68.0±2.4b	83.2±3.9a	57.7±6.7a	77.2±5.2a
D_{15}	16.7±2.0a	18.5±0.1a	70.6±1.5a	82.8±5.0a	57.2±5.3a	72.7±6.2b
D_{20}	16.9±1.8a	16.1±0.5b	68.5±2.2b	80.2±4.0a	41.0±6.1b	71.4±4.7b
P x D	**	NS	NS	NS	NS	NS

P_E and P_L indicate to planting on May 20 and June 20, respectively. *, ** indicate to the significant difference at $P \leq 0.05$ and $P \leq 0.01$, respectively; and "ns" indicates to non-significant difference. Means ± SE in each column followed by the same letter are not significantly different according to Duncan's multiple range test.

Table 6 Effects of planting dates, plant densities and their interaction on electrolyte leakage (EL), free proline and absolute growth rate (AGR) for the two cuts of clitoria plant during 2015 and 2016 growing seasons.

Treatment	EL (%)		Free proline ($mg\ g^{-1}$ DW)		AGR ($g\ d^{-1}$)	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
			2015 season			
Planting date (P)	**	*	**	**	**	NS
Early (P_E)	45.7±2.5b	48.1±1.3b	5.1±0.12a	0.9±0.03b	0.25±0.03a	0.72±0.04a
Late (P_L)	82.3±1.1a	60.6±2.2a	2.6±0.25b	1.4±0.02a	0.15±0.01b	0.75±0.04a
Density (D)	**	**	**	NS	**	**
D_{10}	63.0±9.1b	50.7±3.5b	3.4±0.57c	1.1±0.11a	0.13±0.01c	0.60±0.02c
D_{15}	60.4±9.5c	58.4±4.2a	4.4±0.44a	1.2±0.09a	0.20±0.03b	0.73±0.01b
D_{20}	68.6±6.1a	53.9±1.8b	3.7±0.69b	1.1±0.15a	0.26±0.04a	0.88±0.01a
P x D	**	**	**	*	**	*
			2016 season			
Planting date (P)	**	*	**	**	*	*
Early (P_E)	62.5±2.4b	83.0±0.9b	1.3±0.03b	0.9±0.01b	0.19±0.02a	0.67±0.02a
Late (P_L)	91.8±1.1a	89.1±1.0a	2.7±0.25a	1.1±0.03a	0.15±0.02b	0.44±0.02b
Density (D)	**	NS	**	**	**	**
D_{10}	78.2±7.0a	87.2±1.9a	1.5±0.12b	1.0±0.04c	0.11±0.01c	0.51±0.05b
D_{15}	71.9±7.5b	85.5±2.2a	2.2±0.44a	1.0±0.04b	0.18±0.01b	0.53±0.07b
D_{20}	81.2±5.7a	85.5±1.1a	2.3±0.44a	1.1±0.06a	0.22±0.01a	0.63±0.05a
P x D	NS	NS	**	**	NS	NS

P_E and P_L indicate to planting on May 20 and June 20, respectively. *, ** indicate to the significant difference at $P \leq 0.05$ and $P \leq 0.01$, respectively; and "ns" indicates to non-significant difference. Means ± SE in each column followed by the same letter are not significantly different according to Duncan's multiple range test.

Nutritive value indices

Protein, fiber and NSC contents are considered the most important indices of nutritive value. Planting date was significantly affected these indices of clitoria formerly mentioned in the two cuts of both seasons except for protein content in the 2nd cut of the 2nd season and fiber content in the 2nd cut of the 1st season as presented in (Table 7). The highest protein content (23.7 and 21.2%) in both cuts of the 1st season and fiber content (18.9%) in the 1st cut of the 2nd season were observed with late planting date (P_L), while early planting date (P_E) recorded the highest NSC (46.0 and 49.8%), fiber content (19.0 and 20.6%) in the 1st and 2nd cuts of both seasons, respectively, and protein content (26.3%) in the 1st cut of the 2nd season. Regarding the nutritive value, plant densities caused significant variances for all indices except for fiber content in both cuts in the 1st seasons also protein and fiber in the 1st cut during the 2ndseason. The highest protein (23.1 and 20.3 & 25.6 and 18.3%) was obtained at the higher density D_{10} for both cuts in the 1st and 2nd seasons, respectively, while application of the widest hill spacing namely D_{20}achieved the greatest fiber content (20.8%) for the 2ndcut in the 2nd season. Concerning to NSC, data show that the greatest value (45.6 and 48.9%) was observed when the clitoria plants grown under medium density D_{15} for both cuts of the 1st season, whereas the narrowest hill spacing of D_{10} produced plants contained higher NSC (43.2 and 50.3%) compared to those other densities in both cuts during the 2nd season.

Forage and protein yields

With the exception of CPY in the 1st season, there were statistical differences between planting dates with respect to CFFY and CDFY of clitoria plant in both seasons (Table 8 and Fig.1). Early planting date (P_E) recorded the highest CFFY (20.8 and 21.0 t ha^{-1}) and CDFY (6.4 and 6.6 t ha^{-1}) for both seasons, respectively, alongside CPY (1.27 t ha^{-1}) in the 2nd season. A significant variance due to plant densities was observed for CFFY, CDFY and CPY with respect to the 1st and 2nd growing seasons. Utilization the closest hill

spacing of D_{10} proved to be the most effective density in producing the highest CFFY (23.3 and 23.1 t ha^{-1}), CDFY (7.5 and 7.4 t ha^{-1}) and CPY (1.55 and 1.39 kg m^{-3}) with increases of (43.8 and 47.1%), (47.1 and 54.2%) and (49.0 and 54.7%) compared to the lowest values for these attributes at the widest hill spacing of D_{20} for both seasons, respectively.

Table 7 Effects of planting dates, plant densities and their interaction on protein, fiber and non-structural carbohydrates (NSC) for the two cuts of clitoria plant during 2015 and 2016 growing seasons.

Treatment	Protein (%)		Fiber (%)		NSC (%)	
	1st cut	2nd cut	1st cut	2nd cut	1st cut	2nd cut
	2015 season					
Planting date (P)	**	*	**	NS	**	**
Early (P_E)	22.0±0.08b	18.9±0.22b	19.0±0.04a	18.7±0.15a	46.0±0.12a	49.8±0.18a
Late (P_L)	23.7±0.12a	21.2±0.10a	18.5±0.06b	19.1±0.08a	44.5±0.14b	47.4±0.11b
Density (D)	**	*	NS	NS	**	*
D_{10}	23.1±0.48a	20.3±0.39a	18.7±0.19a	18.7±0.10a	44.9±0.36c	48.4±0.39b
D_{15}	22.7±0.37b	19.7±0.60b	18.8±0.08a	18.9±0.22a	45.6±0.26a	48.9±0.57a
D_{20}	22.8±0.32b	20.1±0.65ab	18.7±0.12a	19.1±0.13a	45.4±0.42b	48.6±0.70b
P x D	*	*	**	NS	**	**
	2016 season					
Planting date (P)	**	NS	**	**	**	NS
Early (P_E)	26.3±0.11a	17.9±0.20a	17.2±0.07b	20.6±0.14a	42.3±0.24b	49.9±0.14a
Late (P_L)	24.6±0.08b	18.0±0.10a	18.9±0.10a	20.2±0.06b	43.4±0.10a	50.3±0.11a
Density (D)	NS	**	NS	**	**	**
D_{10}	25.6±0.35a	18.3±0.08a	18.1±0.50a	20.3±0.07b	43.2±0.02a	50.3±0.14a
D_{15}	25.5±0.34a	17.8±0.16b	18.0±0.33a	20.2±0.09b	42.7±0.24b	49.7±0.16b
D_{20}	25.2±0.46a	17.6±0.20b	18.1±0.31a	20.8±0.19a	42.7±0.49b	50.2±0.13a
P x D	NS	NS	*	NS	**	NS

P_E and P_L indicate to planting on May 20 and June 20, respectively. *, ** indicate to the significant difference at $P \leq 0.05$ and $P \leq 0.01$, respectively; and "ns" indicates to non-significant difference. Means ± SE in each column followed by the same letter are not significantly different according to Duncan's multiple range test.

Table 8 Effects of planting dates, plant densities and their interaction on cumulative fresh forage yield (CFFY), cumulative dry forage yield (CDFY) and cumulative protein yield (CPY) for clitoria plant during 2015 and 2016 growing seasons.

Treatment	CFFY (t ha^{-1})		CDFY (t ha^{-1})		CPY (t ha^{-1})	
	2015	2016	2015	2016	2015	2016
Planting date (P)	**	**	**	**	NS	*
Early (P_E)	20.8±0.9a	21.0±1.2a	6.4±0.3a	6.6±0.4a	1.25±0.067a	1.27±0.076a
Late (P_L)	17.8±1.3b	16.9±0.99b	5.8±0.4b	5.4±0.4b	1.25±0.087a	1.01±0.064b
Density (D)	**	**	**	**	**	**
D_{10}	23.3±0.4a	23.1±1.0a	7.5±0.1a	7.4±0.3a	1.55±0.022a	1.39±0.063a
D_{15}	18.4±0.8b	18.0±1.1b	5.8±0.2b	5.7±0.3b	1.17±0.010b	1.11±0.069b
D_{20}	16.2±0.9c	15.7±0.6c	5.1±0.2c	4.8±0.2c	1.04±0.015c	0.91±0.045c
P x D	**	**	**	NS	**	*

P_E and P_L indicate to planting on May 20 and June 20, respectively. *, ** indicate to the significant difference at $P \leq 0.05$ and $P \leq 0.01$, respectively; and "ns" indicates to non-significant difference. Means ± SE in each column followed by the same letter are not

significantly different according to Duncan's multiple range test.

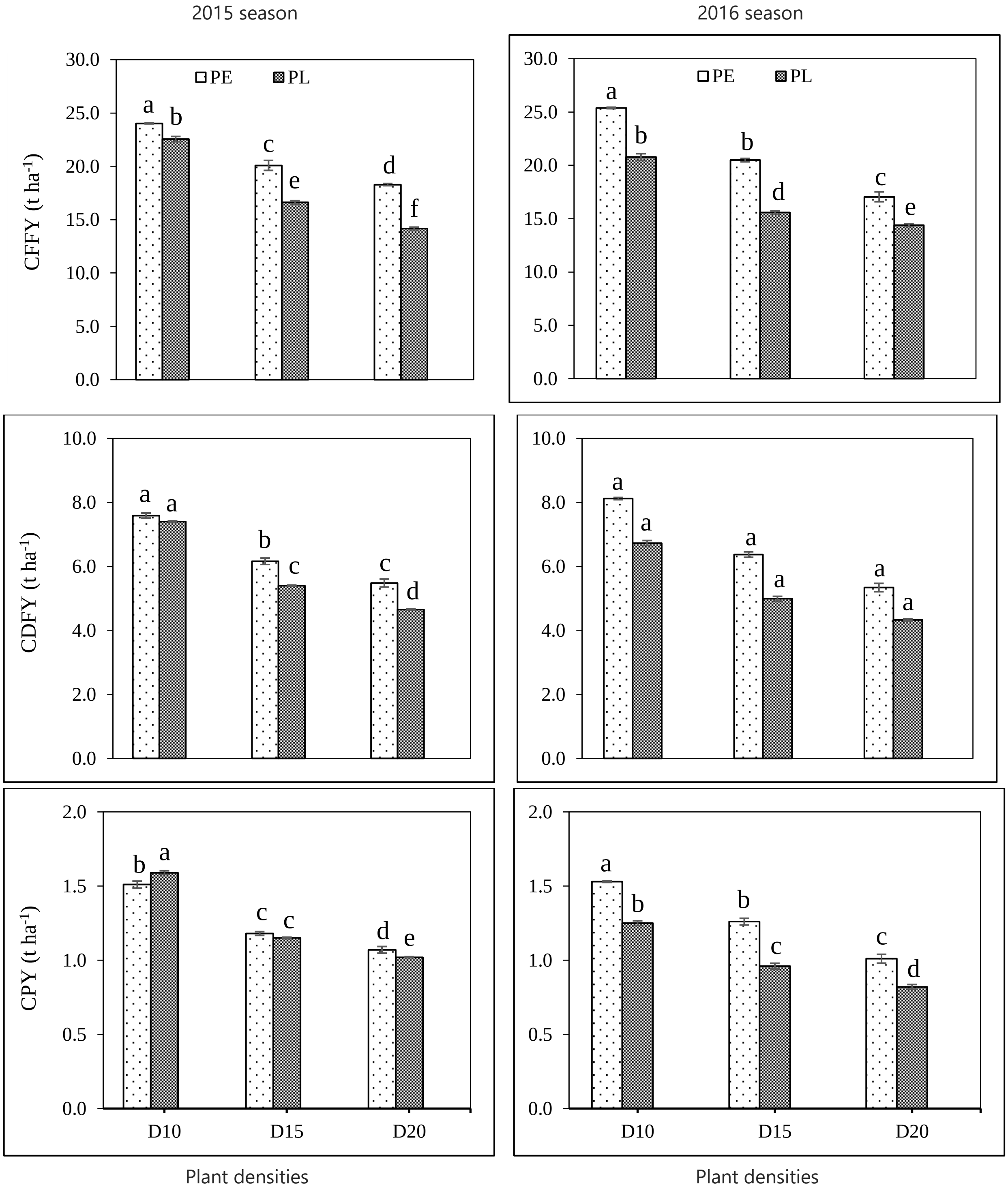

Figure 1

Interactive effects of planting dates and plant densities on CFFY, CDFY and CPY of clitoria during 2015 and 2016 seasons (mean ± SE). P_E and P_L indicate to planting on May 20 and June 20, respectively. Bars labeled with the same letter in each chart are not significantly different according to Duncan's multiple range test.

4. DISCUSSION

A delayed planting of forage clitoria from May 20 to June 20 caused significant decreases in almost all morpho-physiological attributes of clitoria plant in the two cuts in both seasons in both cuts in the two growing seasons. These results are in parallel with those of Abou El-yazied (2011), Singla et al. (2016), and Ramanjaneyulu et al. (2018) on various legume plants. The reductions in aforesaid attributes mainly attributed to that planting at a later date, clitoria plants miss the optimum environmental conditions of optimum planting date such as air temperature, humidity, solar radiation and longer day length as indicated in Table 1. In optimum planting date, these environmental conditions play vital roles in improving plant growth via increase of photosynthetically active radiation that help in rapid seed germination and healthy growth of plant, consequently increasing dry matter accumulation as well as reflecting, positively, in forage yield. Moreover, as delayed planting date from P_E to P_L, the vegetative growing period is shortened. This means a reduction of the accumulation of radiation and deceleration of assimilation rate, particularly during later growing periods during which temperature is reduced and solar radiation is lowered where entering fall season (Table 1). The obtained results are found to be in agreement also with those obtained by Grenz et al. (2005) who mentioned that delayed sowing faba bean by month resulted in a shorter growing period, lower growth rates, and consequently a reduction in the final dry weight of shoots by 10 to 34%. Concerning to physiological attributes, delaying planting date is considered an important environmental stress. At the cellular level, plants attempt to decrease the negative effects of this stress by decreasing their assimilation rate and also by inhibiting the biochemical processes and growth, causing a general decrease in RWC and MSI, and an increase in EL due to injury of cell membrane caused by stress therefore undesirable increase of cell permeability. In addition, increasing free proline content as an osmolyte compound to keep cellular osmotic homeostasis, all of these are consistent confirmed with our results in (Tables 5 and 6) by exposing clitoria plants to delayed planting date for one month. Similar trend of findings were reported by Zlatev and Yordanov (2004) on bean plant, Talukdar (2011) on clitoria and Ghanepour et al. (2015) on common bean.

Our previous results revealed that, once the plant density has been increased from D_{20} to D_{15} or D_{10}, significant increase was detected regarding PH of clitoria (Kumalasari et al. 2017), this probably be due to high rate of stem elongation which is related to the competition among plants for pre-empting light, water and nutrients supplies in addition to shading effect in high plant density treatment which encouraged vertical growth of the plants compared to those in low plant density (Craine and Dybzinski 2013). However, significant or insignificant decreases were observed as increased plant density from D_{20} to D_{15} or D_{10} in terms of NL/P, NB/P, PDW and AGR in both growing seasons. These results were previously reported for other crops (Deka et al. 2015; Kumalasari et al. 2017). We suggest two integral interpretations for these decreases. First, by reducing the plant spacing in high plant density, the plants displayed a stronger apical dominance in search of solar radiation, thus producing fewer lateral branches compared to those in wider plant spacing (Streck et al. 2014). Second, the early canopy closure for plants grown under narrow plant spacing reducing their ability for intercept solar radiation that reach to the leaves and reduce their chlorophyll content and weakening photosynthesis rate and hence undermining plant growth (Mattera et al. 2013). Worth mentioning that, the thinner stems of plants grown under high plant density could provide faster field drying at harvest and may reduce the coarse stem sections by animal at feeding (Hintz et al. 1992). Also, findings obviously referred to clitoria plants grown under high plant density have higher LAI than those under low plant density (Mattera et al. 2013; Mojaddam and Noori 2015). The higher LAI in closer plant spacings might be due to an enhanced number of leaves produced per unit area under this condition.

At the cellular level, the results revealed that the RWC and MSI decreased but EL and free proline increased as intra-ridge spacing among hills increased (low plant density) particularly in the 1st cutting. These results could be due to the greater soil evaporation relative to crop evapotranspiration under widening plant spacing within the ridge before the fully establishment of plant canopy that shades the soil surface, depending on air temperature and relative humidity particular in early stages of clitoria plant. Thus, early vigorous growth for plants might be an effective way for establishing canopy cover quicker to minimize soil moisture evaporation. On the other side, the RWC and MSI increased while EL and free proline decreased with decreasing of intra-ridge spacing among hills (high plant density) especially in the 2nd cut of clitoria plant in the present study, this finding supported by Suresh et al. (2013) on pigeon pea plant. These results probably ascribed to improving soil and leaf water status resulting from the decreasing of evaporation from the soil surface due to near complete coverage by plant canopy under narrow hill spacings with more uniform planting distribution and therefore increase dry matter content of plant.

In spite of the increased of protein in forage clitoria, fiber and NSC decreased when planting date delayed in the two cuts of both seasons. This result has been reported by (Morsy and Awadalla, 2017) on clitoria. Increasing of protein in clitoria produced from P_L might be due to that the clitoria herbage produced from delayed planting date was less mature than that obtained from the early planting date (Testa et al. 2011). On the other side, the reduction in fiber and NSC contents in clitoria induced by delaying planting date may be due to its inhibitory effect on photosynthetic activities, leaf photosynthetic pigment concentrations and the activity of RuBisCO enzyme causing decreases in all carbohydrates forms (Abu-Muriefah 2013).

The forage protein in both cuts under high density considerably increased as previously reported by Stević et al. (2012) on sainfoin and Helmy et al. (2015) on cowpea. This result in our study is probably associated with increasing LSR. However, the increments of fiber or NSC as plant density decreased might be ascribed to the vigorous growth for root system under wider row spacings which enhances water and nutrients uptake that would result in a greater canopy leaf area development and greater light interception, consequently increase the accumulate of dry matter components in plant (Ayub et al. 2011).

The obvious reduction in productivity of clitoria in terms of forage and protein induced by delaying planting date has been reported in several works of Keogh et al. (2012), Sim et al. (2015), Abd El-Mageed et al. (2017), and Zhang et al. (2017) carried out by using different legume plants. The superiority of early planting date for productivity of clitoria (i.e., CFFY, CDFY, and CPY) may have resulted from the fact that early planted crop had sufficient longer vegetative period and better utilization of water, climatic elements and soil nutrients. Moreover, the reduction in forage yield in late-planted clitoria might be related to their obvious reductions in vegetative growth attributes (e.g., PH, NL/P, NB/P, LA/P, PDW, and LSR) resulting in lower dry matter production.

our observations indicate that, the higher forage and protein yields of clitoria due to high population density were previously reported for various crops such as narbon vetch (Yilmaz 2008), columbus grass (Olanite et al. 2010) and Sudan grass (Awad et al. 2013). With regard to forage yield, although the PDW for individual plant decreased as the plant population density per unit area increased, the high number of plants per unit area compensated for the reduction in weight per plant, resulting in an increase in the CDFY per area when the plant density increased (Stanisavljević et al. 2012). The highest yields (i.e., CFFY, CDFY and CPY) when the P_E x D_{10} interaction was applied as illustrated in Fig. 1. This result provides clear evidence that the strong association between proper planting date and optimal plant density and its direct effect in achieving high productivity of forage and protein from clitoria (Feyzbakhsh et al. 2015; Bajaj et al. 2008). Where early planting date (20 May) under experiment site conditions is necessary to get optimum crop performance which probably makes crop more tolerance to high plant density (33.33 plants m^{-2}). On the other, increased plant density may also alleviate the adverse effects of delayed planting date by producing more dry matter per unit area.

5. CONCLUSION

Selection of the suitable planting time ensures providing an optimal climate condition for the crop in each stage to endure higher plant density via vigorous growth. Results of the present study showed that morpho-physiological parameters, total chl., RWC, MSI, AGR, fiber and NSC contents, CFFY, CDFY, and CPY improved with early planting date but become limited when delaying planting date of clitoria. However, delaying planting date showed increases in EL, free proline, and forage protein content. Regarding to plant density, the results revealed that, the NL/P, NB/P, PDW, total chl., RWC, EL, free proline, and AGA increased but PH, LAI, LSR, and MSI decreased as intra-ridge spacing among hills increased from D_{10} (high density) or D_{15} (medium density) to D_{20} (low density). Furthermore, the greatest forage protein was obtained at D_{10} (high density), however the plant density by means of changing hill spacing seemed to be a fluctuate factor concerning to fiber and NSC parameters. However, the highest CFFY, CDFY, and CPY were recorded when the narrowest hill space of D_{10} (high density) was implemented. Based on the results of the current investigation, it could be reported that the combination of P_E x $D_{10}$is the most suitable for producing great forage yield and acceptable nutritive value indices from clitoria as a recently introduce plant in newly reclaimed soils as an arid environment such El-Fayoum province conditions in Egypt.

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Conflict of interest

No possible conflict of interest was informed by the authors.

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